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Product Information

ACCUSPIN™ Tubes

Catalog Numbers **A2055 and A1805**
Store at Room Temperature

Product Description

ACCUSPIN™ Tubes are intended for use with the density gradient separation medium Histopaque®-1077 in the isolation of lymphocytes and other mononuclear cells. The ACCUSPIN Tube is a specially designed polypropylene centrifuge tube with two chambers separated by a porous high density polyethylene barrier (frit).

Separation of lymphocytes and other mononuclear cells from whole blood and bone marrow using density gradient separation media is based on a published method.[1] Histopaque-1077 is suitable for human lymphocyte antigen (HLA) typing[2] and as the initial isolation step prior to enumeration of T, B, and 'null' lymphocytes.[3] It may also be employed in the preparation of pure lymphocyte suspensions for cell culture and cytotoxicity assays.[4]

ACCUSPIN Tubes consist of radiation sterilized polypropylene tubes fitted with a high density polyethylene frit.

ACCUSPIN Tubes, 50 mL capacity Catalog No. A2055	10 each
ACCUSPIN Tubes, 12 mL capacity Catalog No. A1805	20 each

Reagents and Equipment Required but Not Provided

- Histopaque-1077, Catalog No. 10771
- Centrifuge (swinging bucket rotor) capable of generating 1,000 × *g*
- Centrifuge tubes for washing mononuclear cells
- Isotonic phosphate buffered saline solution or appropriate cell culture medium

Precautions and Disclaimer

ACCUSPIN Tubes are for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Specimen Collection - Collect blood in preservative-free anticoagulant (EDTA or heparin) or use defibrinated blood. For best results, blood should be processed within 2 hours.

On occasion, it may be necessary to dilute the blood sample 3 to 5-fold, depending on absolute cell numbers. A similar volume of prediluted blood may be used or the blood sample may be diluted directly in upper chamber of the ACCUSPIN tube (see Procedure, step 4). This is appropriate for specimens with hematocrits above normal.

Storage/Stability

Store ACCUSPIN Tubes at room temperature. In the event the integrity of the radiation sterilized tube is compromised (e.g., cracked or loose cap) sterility is not guaranteed.

Procedure

Following the addition of Histopaque-1077 to the ACCUSPIN Tube, a brief centrifugation places the Histopaque-1077 below the frit. The blood sample can be added to the top chamber of the tube without risk of mixing with the Histopaque-1077 in the lower chamber under the frit. On subsequent centrifugation the whole blood descends through the frit to contact with the Histopaque-1077. The elements of greater density displace a volume of Histopaque-1077 above the frit giving a clear separation of the blood components. The erythrocytes aggregate and the granulocytes become slightly hypertonic, increasing their sedimentation rate, resulting in pelleting at the bottom of the ACCUSPIN Tube. Lymphocytes and other mononuclear cells, e.g., monocytes, remain at the plasma/Histopaque-1077 interface. This dense band of mononuclear cells may be collected by pouring off the contents of the upper chamber or by means of a pipette. Erythrocyte contamination is avoided due to the barrier between the chambers.

1. Bring Histopaque-1077 to room temperature. Protect from light.
   Note: Failure to bring Histopaque-1077 to room temperature prior to use may cause limited recovery of mononuclear cells.

2. Pipette Histopaque-1077 into the upper chamber of each ACCUSPIN Tube.
   a. Use 3 mL of Histopaque-1077 for ACCUSPIN Tube, 12 mL capacity, Catalog No. A1805.
   b. Use 15 mL of Histopaque-1077 for ACCUSPIN Tube, 50 mL capacity, Catalog No. A2055.

3. Centrifuge at 800 × *g* for 30 seconds at room temperature. The Histopaque-1077 will now be in the chamber below the frit.

4. Freely pour the blood sample into the upper chamber of each ACCUSPIN Tube.
   a. Use 3–6 mL of blood sample for ACCUSPIN Tube, 12 mL capacity, Catalog No. A1805.
   b. Use 15–30 mL of blood sample for ACCUSPIN Tube, 50 mL capacity, Catalog No. A2055.
   Note: Use of volumes of prediluted or whole blood other than those recommended may result in decreased recovery.

5. Centrifuge at 1,000 × *g* for 10 minutes at room temperature. Alternatively, centrifuge at 800 × *g* for 15 minutes at room temperature. Centrifugation at lower temperatures, such as 4 °C, may result in cell clumping and poor recovery.
   Notes: Occasionally a frit may become dislodged during centrifugation. If this occurs, do not attempt to pour off the contents of tube to collect the mononuclear cells. Instead, gently remove frit with sterilized forceps, or tilt the frit with a pipette and then collect the mononuclear cells.

   To remove all contaminating platelets, a second centrifugation in a 4–20% sucrose gradient layered over Histopaque-1077 can be performed. The sucrose gradient will effectively isolate the platelets, while the mononuclear cells will penetrate to the Histopaque-1077 layer.

6. After centrifugation, carefully aspirate the plasma layer with a Pasteur pipette to within 0.5 cm of the opaque interface containing the mononuclear cells. Properly dispose of the plasma layer.
   Note: Failure to remove the excess supernatant may result in contamination of the mononuclear band with plasma proteins.

7. Carefully transfer the opaque interface above the frit with a Pasteur pipette into a clean conical centrifuge tube.
   Note: Removal of Histopaque-1077 with the mononuclear band increases granulocyte contamination from residual granulocytes, which may remain at the mononuclear interface.

8. Wash the cells by adding 10 mL of isotonic phosphate buffered saline or appropriate cell culture medium, and mix the cells by gently drawing in and out of a Pasteur pipette.

9. Centrifuge at 250 × *g* for 10 minutes at room temperature.

10. Aspirate the supernatant and discard.

11. Resuspend cell pellet with 5 mL of isotonic phosphate buffered saline solution or appropriate cell culture medium, and mix by gently drawing in and out of a Pasteur pipette.

12. Centrifuge at 250 × *g* for 10 minutes.

13. Repeat steps 10, 11, and 12, discard supernatant and resuspend cell pellet in 0.5 mL of isotonic phosphate buffered saline solution or appropriate cell culture medium.

Erythrocytes and granulocytes should pellet to the bottom of the ACCUSPIN tube. Mononuclear cells should band at the interface between the Histopaque-1077 and the plasma. If observed results vary from expected results, please contact Sigma-Aldrich Technical Service for assistance.

References

1. Boyum, A., Separation of leukocytes from blood and bone marrow. Scand. J. Clin. Lab. Invest., **21** (Suppl 97), 77 (1968).
2. Amos, D.B., and Pool, P., HLA typing in Manual of Clinical Immunology, Rose, N.R., and Friedman, H., eds., American Society for Microbiology, (Washington, DC: 1976) pp. 797-804.
3. Winchester, R.J., and Ross, G., Methods for enumerating lymphocyte populations in Manual of Clinical Immunology, Rose, N.R., and Friedman, H. eds., American Society for Microbiology, (Washington, DC: 1976) pp. 64-76.
4. Thorsby, E., and Bratlie, A., A rapid method for preparation of pure lymphocyte suspensions. Histocompatibility Testing, Terasaki, P.I., ed., 665-666 (1970).

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